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UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

Ex parte KAREN UHLMANN, PETER NURNBERG, and
ANJA BRINCKMANN

Appeal 2010-009243
Application 10/823,784
Technology Center 1600

Before DEMETRA J. MILLS, ERIC GRIMES, and LORA M. GREEN, *Administrative Patent Judges*.

GRIMES, *Administrative Patent Judge*.

DECISION ON APPEAL[1]

This is an appeal under 35 U.S.C. § 134 involving claims to an assay for determining whether a nucleotide in a nucleic acid is methylated. The

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[1] The two-month time period for filing an appeal or commencing a civil action, as recited in 37 C.F.R. § 1.304, or for filing a request for rehearing, as recited in 37 C.F.R. § 41.52, begins to run from the "MAIL DATE" (paper delivery mode) or the "NOTIFICATION DATE" (electronic delivery mode) shown on the PTOL-90A cover letter attached to this decision.

Examiner has rejected the claims as obvious in view of the prior art. We have jurisdiction under 35 U.S.C. § 6(b). We affirm.

STATEMENT OF THE CASE

The Specification discloses that "[s]odium bisulfite-treatment of DNA converts unmethylated cytosine into uracil, which is subsequently amplificated as thymine in a PCR. Methylated cytosine, however, is non-reactive and remains detectable as a cytosine." (Spec. 1.)

Claims 1-5, 7-20, and 22-39 are on appeal. Claims 1 and 10 are representative and read as follows:

1. A method for detecting the methylation status of a nucleotide at a predetermined position in a nucleic acid molecule comprising:

(a) treating a sample comprising said nucleic acid molecule in an aqueous solution with an agent suitable for the conversion of said nucleotide if present in

(i) methylated form; or

(ii) non-methylated form

(b) to pair with a nucleotide normally not pairing with said nucleotide prior to conversion;

(c) amplifying said nucleic acid molecule treated with said agent via at least one amplification primer to produce an amplification product and converting said amplification product into single stranded amplified nucleic acid molecules, wherein said at least one amplification primer is detectably labeled with a detectable label that forms an anchor for removal of said single stranded amplified nucleic acid molecules to generate a single stranded amplified nucleic acid molecule;

(d) real-time sequencing said single stranded amplified nucleic acid molecule; and

(e) detecting whether said nucleotide is methylated or not methylated at said predetermined position in the sample.

10. The method of claim 1, further comprising calculating a frequency of methylated nucleotides from results of said real-time sequencing.

The claims stand rejected under 35 U.S.C. § 103(a) as follows:

- Claims 1-5, 7-9, 11, 12, 19, 20, 22-24, 26-33, and 36 based on Uhlmann[2] and Nyren[3] (Answer 3);
- Claims 10, 25, 34, and 39 based on Uhlmann, Nyren, and Sylvan[4] (Answer 9);
- Claims 12-16, 18, and 38 based on Uhlmann, Nyren, and Herman[5] (Answer 7);
- Claim 17 based on Uhlmann, Nyren, Herman, and Feinberg[6] (Answer 8);
- Claim 35 based on Uhlmann, Nyren, and Laird[7] (Answer 11); and
- Claim 37 based on Uhlmann, Nyren, and Hyman[8] (Answer 12);

I.

Issue

The Examiner has rejected claims 1-5, 7-9, 11-20, 22-24, 26-33, and 35-38 as obvious in view of Uhlmann and Nyren, alone or combined with additional references. Because Appellants present the same argument with respect to each of these rejections (Appeal Br. 10-20, 22), we will consider them together. In addition, with the exception of claim 32, which is

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[2] Karen Uhlmann et al., *Changes in methylation patterns identified by two-dimensional DNA fingerprinting*, 20 ELECTROPHORESIS 1748-1755 (1999)
[3] Nyren, US 6,258,568, July 10, 2001
[4] Sylvan, US 7,078,168, July 18, 2006
[5] Herman et al., US 5,786,146, July 28, 1998
[6] Feinberg, US Patent Application Publication 2003/0232351, Dec. 18, 2003
[7] Laird et al., US Patent Application Publication 2002/0086324, July 4, 2002
[8] Hyman, US 5,602,000, Feb. 11, 1997

addressed below, the claims were not argued separately from claim 1 and therefore stand or fall with that claim. 37 C.F.R. § 41.37(c)(1)(vii).

The Examiner finds that Uhlmann discloses the claimed method except that Uhlmann does not use a labeled amplification primer that forms an anchor and Uhlmann does not sequence its amplified nucleic acids using a real-time sequencing method (Answer 4). The Examiner finds that Nyren discloses a real-time sequencing method that includes amplifying DNA using a primer that carries a functional group such as biotin that allows subsequent immobilization (*id.* at 4-5). The Examiner concludes that it would have been obvious to modify Uhlmann's method by using Nyren's sequencing method, including PCR using a biotin-labeled amplification primer, because Nyren discloses that its method allows "DNA to be sequenced simply and rapidly while avoiding the need for electrophoresis and use of harmful radiolabels" (*id.* at 5-6).

Appellants contend that the claims require "that treatment of the sample, e.g., with bisulfite, takes place in '<u>an aqueous solution</u>,'" while Uhlmann's bisulfite treatment takes place in agarose beads (Appeal Br. 14). Appellants also contend that modifying Uhlmann's method with Nyren's sequencing method would not have been obvious because Uhlmann was primarily interested in precision and the "prospect of an automatic approach . . . as described by Nyren, would be, if at all, at best be of secondary importance" (*id.* at 18).

The issue with respect to this rejection is: Does the evidence of record support the Examiner's conclusion that Uhlmann and Nyren would have made obvious a method meeting the limitations of claim 1 on appeal?

Findings of Fact

1. Uhlmann discloses "determin[ing] the methylation state of distinct nucleotides within a human DNA fragment of interest using the bisulfite approach. This technique is based on sodium bisulfite-mediated conversion of nonmethylated cytosines to uracil." (Uhlmann 1749, left col.) The uracil is amplified as thymine (T) is a PCR reaction (*id.* at 1750, Figure 1).

2. Uhlmann discloses that after the bisulfite-modified DNA is amplified by PCR, cloned, and sequenced, "this method reveals the methylation status of distinct CpGs in individual DNA strands" (*id.*).

3. Nyren discloses a real-time DNA sequencing method (Nyren, col. 5, ll. 61-64) in which the "sample DNA may be amplified," (*id.* at col. 8, l. 9) and "[i]mmobilisation of the amplified DNA may take place as part of PCR amplification itself . . . or alternatively one or more of the PCR primers may carry a functional group permitting subsequent immobilisation, eg. a biotin or thiol group" (*id.* at col. 8, ll. 21-26.)

4. Nyren discloses that its "assay technique is very simple and rapid, thus making it easy to automate by using a robot apparatus where a large number of samples may be rapidly analysed" (*id.* at col. 8, ll. 61-63).

5. Nyren refers to its sequencing method as "PyroSequencing" (*id.* at col. 20, ll. 25-53).

6. The Specification discloses that "[m]ost preferably, real-time sequencing is performed by the pyrosequencing method" (Spec. 14).

7. The Specification states that "[t]he aqueous solution may be water such as distilled water, a buffered solution such as a phosphate buffered

solution or buffered solution other than a phosphate buffered solution, to name some important examples" (*id.* at 6).

8. The Specification provides an example of the disclosed method in which genomic DNA was mixed with "4% low melting agarose . . . and a single bead with a final volume of 20 µl was formed in prechilled mineral oil. Bisulfite conversion was performed. . . . TE-buffer (pH 8) was used for washing the beads. . . . For amplification with PCR the agarose beads were diluted with 180 µl HPLC H_2O." (*Id.* at 21.)

Analysis

Uhlmann discloses a method of determining the methylation status of a nucleotide in a nucleic acid by treating the nucleic acid with sodium bisulfite, which converts unmethylated cytosine to uracil; the converted nucleotide therefore would pair with a nucleotide it would not have paired with before conversion. Uhlmann's method also amplifies and sequences the converted nucleic acid and detects whether a given nucleotide is methylated in the sample nucleic acid. Uhlmann's method therefore includes steps (a), (b), and (e) of claim 1.

Nyren discloses a real-time sequencing method, as recited in step (d) of claim 1, in which a PCR primer used to amplify the target DNA can include a functional group permitting immobilization of the amplified DNA, which meets the limitations of step (c) of claim 1 requiring amplifying using a primer having a detectable label that forms an anchor.

We agree with the Examiner that it would have been obvious to modify Uhlmann's method by using Nyren's labeled-primer amplification and real-time sequencing method, rather than the time-consuming

amplification, cloning, and gel sequencing disclosed by Uhlmann, because Nyren discloses that its method is simple, rapid, and easy to automate. "[A]n implicit motivation to combine exists not only when a suggestion may be gleaned from the prior art as a whole, but when the 'improvement' is technology-independent and the combination of references results in a product or process that is more desirable, for example because it is . . . faster . . . or more efficient." *Dystar Textilfarben GmbH & Co. Deutschland KG v. C.H. Patrick Co.*, 464 F.3d 1356, 1368 (Fed. Cir. 2006).

Appellants argue that claims 1 and 12, and the claims that depend on them, require the bisulfite treatment step to take place in aqueous solution, while Uhlmann describes carrying out this step on DNA suspended in agarose beads (Appeal Br. 14). On a similar note, Appellants argue that substituting Nyren's PCR and sequencing steps for those of Uhlmann "leaves Uhlmann '99's DNA that is to be subjected to Nyren's PCR and sequencing in the agarose beads (1.7% low melting agarose . . .). This might raise the question as to whether the relative[ly] complex sequencing reaction of Nyren that follows his PCR could be performed in such an environment." (*Id.* at 15.)

These arguments are not persuasive. The Specification provides some non-limiting examples of aqueous solutions (FF 7), which do not include agarose gel, but the only working example of the Specification carries out the bisulfite treatment step on DNA that is suspended in an agarose bead (FF 8), just as described in Uhlmann. Since the Specification does not provide a definition of "aqueous solution" that excludes agarose beads, and the Specification exemplifies the disclosed method using DNA in an agarose

bead, the broadest reasonable interpretation of "aqueous solution" that is consistent with the Specification encompasses DNA suspended in agarose beads.

Appellants' argument that it is unclear whether Nyren's method would work using DNA suspended in agarose beads is also unpersuasive. Uhlmann discloses that agarose beads containing bisulfite-converted DNA can be used directly in a PCR amplification, without extracting the DNA from the beads (Uhlmann 1750-1751). Logically, this makes sense: the DNA was suspended in "low melting agarose" and the PCR process subjects the samples to repeated cycles of denaturation at 95° C; it is reasonable to expect that heating low-melting agarose to 95° C would melt the agarose and release the previously suspended DNA. Appellants have pointed to no evidence to show that Nyren's PCR and sequencing procedure would not work starting with DNA suspended in agarose beads.

Appellants also argue that it would not have been obvious to use Nyren's real-time sequencing procedure with Uhlmann's process because Uhlmann was primarily concerned about precision, not speed (Appeal Br. 17-18).

This argument is also unpersuasive. Obviousness is determined from the viewpoint of a hypothetical person of ordinary skill in the art. Although Uhlmann may have been satisfied to sacrifice speed to ensure sequencing accuracy, others of ordinary skill in the art would appreciate the advantage of being able to sequence multiple samples quickly and automatically using a robot apparatus as disclosed by Nyren. A person of ordinary skill in the art

would therefore have considered it obvious to combine the references, even if Uhlmann herself would not.

With respect to claim 32, Appellants argue that "[t]he Examiner has not provided any showing for the element 'determining the amount of said nucleotide pairing with said new nucleotide pairing partners' as set forth in the claim and thus has not provided a complete analysis" (Appeal Br. 14).

The Examiner, however, reasons that Nyren's method involves detecting the release of pyrophosphate when a given dNTP is incorporated into a nascent DNA strand being formed on the single-stranded target DNA: "Since Nyren teaches determining the amount of incorporated nucleotides which are base pairing with the DNA strand that is being sequence[d], Nyren teaches the step of determining the amount of nucleotide pairing with the nucleotide pairing partners" (Answer 14). Appellants filed a Reply Brief but did not point out any flaw in the Examiner's claim interpretation or her conclusion that the claim language reads on Nyren's procedure. Appellants' argument in the Appeal Brief therefore does not persuade us that the Examiner erred in rejecting claim 32 based on Uhlmann and Nyren.

Conclusion of Law

The evidence of record supports the Examiner's conclusion that Uhlmann and Nyren would have made obvious a method meeting the limitations of claim 1 on appeal.

II.

Issue

The Examiner has rejected claims 10, 25, 34, and 39 as obvious in view of Uhlmann, Nyren, and Sylvan. The Examiner finds that "Sylvan teaches a method of determining the frequency of an allele in a population of nucleic acid molecules" based on a pattern of nucleotide incorporation in a pyrosequencing reaction (Answer 10). The Examiner concludes that it would have been obvious "to have modified the method of Uhlmann and Nyren by further calculating the frequency of methylated nucleotides from the results of the pyrosequencing as suggested by Sylvan" (*id.* at 11).

Appellants contend that "Sylvan makes no reference to the methylation status of his population of nucleic acid molecules and certainly not 'calculating a frequency of methylated nucleotides' as required by claims 10 and 25" (Appeal Br. 20). Appellants also contend that Sylvan would not make obvious the degree of accuracy required by claims 34 and 39 (*id.* at 21-22).

The issues with respect to this rejection are: Does the evidence of record support the Examiner's conclusions that it would have been obvious to combine Sylvan's calculation method with the method made obvious by Uhlmann and Nyren, and that the references also would have made obvious the limitations of claims 34 and 39?

Additional Findings of Fact

9. Uhlmann discloses that a "reduced level of global DNA methylation is a common finding in a variety of cancer types. . . . Therefore,

the use of changes in the methylation pattern as a prognostic marker in cancer is under investigation." (Uhlmann 1753.)

10. Sylvan discloses a "method of determining the frequency of an allele in a population of nucleic acid molecules," based on a pattern of nucleotide incorporation (Sylvan, abstract).

11. Sylvan discloses detecting allele frequencies of 5% or less (*id.* at Figs. 4a-4c).

12. Sylvan discloses that its method "is advantageously based on a method of 'sequencing-by-synthesis'" (*id.* at col. 3, ll. 27-28) such as Pyrosequencing™ (*id.* at col. 3, l. 50).

13. Sylvan discloses that "by identifying how much of each nucleotide is incorporated at the polymorphic site in a primer extension reaction, it is possible to calculate the frequency of each allele" (*id.* at col. 10, ll. 53-56).

14. Sylvan describes how to calculate the frequency with which a given nucleotide occurs at a given position (*id.* at col. 22, ll. 39-67).

15. Sylvan discloses that its method

> is particularly advantageous in studies of mutations associated with cancer. In this case, the population is a sample of cells removed from a patient. . . . The population can then be scanned for SNPs which are associated with diseased state in the patient, giving patient-specific information on the disease-associated allele, and the frequency of that allele in a population of cells. This type of information could be invaluable in the treatment of cancer, by aiding diagnosis and prognosis.

(*Id.* at col. 21, ll. 53-65.)

Analysis

Claim 10 depends on claim 1 and adds the limitation of "calculating a frequency of methylated nucleotides from results of said real-time sequencing." Uhlmann and Nyren, as previously discussed, would have made the method of claim 1 obvious to a person of ordinary skill in the art.

Uhlmann discloses that bisulfite treatment causes conversion of unmethylated cytosines (but not methylated cytosines) to thymine in a resulting PCR product (FF 1). Uhlmann also discloses that reduced methylation is common in cancer cells and that changes in methylation pattern may be a useful prognostic marker (FF 9).

Sylvan discloses a method of using the results of real-time sequencing (e.g., pyrosequencing) to calculate the frequency of specific alleles at specific positions (FFs 10, 12-14), and suggests using its method to determine the frequency of cancer-associated alleles in patient samples to aid in diagnosis and prognosis (FF 15).

We agree with the Examiner that it would have been obvious to modify the method made obvious by Uhlmann and Nyren by calculating the frequency of methylated nucleotides (i.e., the frequency of cytosines not converted to thymine by bisulfite treatment) because Uhlmann discloses that reduced methylation is associated with cancer and may be useful in prognosis, and Sylvan suggests using its method to calculate the frequency of cancer-associated alleles in a patient's DNA.

Appellants argue that "Sylvan makes no reference to the methylation status of his population of nucleic acid molecules" (Appeal Br. 20). This argument is not persuasive because Uhlmann discloses that bisulfite

treatment change a nucleotide position from unmethylated cytosine to thymine in a resulting PCR product, and a person of ordinary skill in the art would have considered it obvious to use Sylvan's method to calculate the frequency of cytosine versus thymine as a method of determining methylation frequency, for the reasons discussed above.

With respect to claims 34 and 39, the Examiner reasons that "Sylvan teaches that for SNPs SNPE1.5, SNPE7.5 and SNPE4.5 (See Figs 4a-c) it is expected that allele frequencies of 5% with a standard deviation of not more than 1% can be detected" (Answer 10). Although Appellants "direct the attention to Fig. 6 of Sylvan" (Appeal Br. 21), they do not explain how that figure supports their position or explain why Figures 4a through 4c do not support the Examiner's conclusion that it would have been obvious to modify Sylvan's method, if necessary, to calculate the recited allele frequencies.

Conclusion of Law

The evidence of record supports the Examiner's conclusion that it would have been obvious to combine Sylvan's calculation method with the method made obvious by Uhlmann and Nyren. The evidence of record also supports the Examiner's conclusion that the references would have made obvious the limitations of claims 34 and 39.

SUMMARY

We affirm all of the rejections on appeal.

TIME PERIOD FOR RESPONSE

No time period for taking any subsequent action in connection with this appeal may be extended under 37 C.F.R. § 1.136(a).

AFFIRMED

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